Cancer Letters 448 (2019) 144–154

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Aptamer-functionalized liposomes for targeted cancer therapy

Seyedeh Alia Moosavian[a], Amirhossein Sahebkar[b,c,d,*]

[a] Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[d] School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Keywords:
Chemotherapy
Drug delivery
Liposome
Aptamer

ABSTRACT

Accumulation of chemotherapeutic agents in the tumor tissue while reducing adverse effects and drug resistance are among the major goals in cancer therapy. Among nanocarriers, liposomes have been found to be more effective in the passive targeting of cancer cells. A promising recent development in targeted drug delivery is the use of aptamer-functionalized liposomes for cancer therapy. Aptamer-targeted liposomes have enhanced uptake in tumor cells as shown *in vitro* and *in vivo*. Here, we discuss the aptamer-functionalized liposome platforms and review functionalization approaches as well as the factors affecting antitumor efficiency of aptamer-targeted liposomal systems. Finally, we provide a comprehensive overview of aptamer-targeted liposomes based on the molecular targets on the surface of cancer cells.

1. Introduction

Designing selective delivery systems in cancer treatment is an upward trend in pharmaceutical technology. Most cancer therapeutic agents have a narrow therapeutic index and a wide off-target toxicity [1]. Hence, selective targeting to the tumor site is a crucial factor to improve therapeutic efficacy and minimize the adverse effects of anticancer agents. Employing nanoparticles as delivery systems has been the subject of extensive research during the past decades [2,3]. Nanoparticles can be engineered to transport and release drugs in the vicinity of target cells. Liposomes are one of the most widely used nanoparticles for the delivery of anti-cancer agents owing to their promising characteristics such as high biocompatibility, low toxicity, lack of immunogenicity, high stability and high drug loading efficiency [4,5]. Liposomes were first described by Bangham and colleagues in the 1960s [6]. They are microscopic lipid bilayers consisting of an aqueous core which are able to carry either hydrophilic or hydrophobic drugs [7]. The first FDA-approved liposomal formulation was PEGylated liposomal doxorubicin, Doxil®, which is indicated for the treatment of solid tumors. Encapsulation of doxorubicin in PEGylated liposomes improves pharmacokinetic profile of doxorubicin [8]. Liposomal encapsulation increases the half-life and delays the clearance of doxorubicin. Moreover, cardiotoxicity of the liposomal formulation is less than the free drug [9]. Liposomal formulations of cisplatin, daunorubicin, cytarabine and vincristine are other examples of liposomal products approved for cancer therapy [10]. All these liposomal formulations deliver their cargo to the tumor site by the "enhanced permeability and retention" (EPR) effect mechanism. The discovery of EPR in solid tumors is considered as a landmark advance that has enabled fabrication of nanoparticulate delivery systems for cancer therapy [11]. The mechanism of EPR effect has been discussed in details extensively [12–14]. Two parameters are crucial in the tumor accumulation of liposomal formulations: properties of tumor microenvironment and physicochemical characteristics of liposomes. Passive targeting is based on the body's natural response to physicochemical characteristics of the delivery system [1]. While passive targeting has literally improved pharmacokinetic properties of chemotherapeutic agents, it has also introduced new challenges to the treatment process. Taking Doxil® as an example, although tumor accumulation of doxorubicin is improved, only a modest increase in treatment efficacy can be observed [15–20]. This challenge also applies to liposomal formulations of other chemotherapeutic agents such as cisplatin [21]. Furthermore, the slow release of drug from liposome can lead to drug resistance [15]. It has been reported that sterically stabilized liposomes are not effective formulations for the treatment of many cancers like sarcoma, non-small cell lung cancer or hepatoma [22]. In spite of reduced cardiotoxicity of doxorubicin, studies have shown that the use of Doxil® can lead to newfangled side effects including hand-foot syndrome, myelosuppression

* Corresponding author. Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, P.O. Box: 91779-48564, Iran.
E-mail addresses: sahebkara@mums.ac.ir, amir_saheb2000@yahoo.com (A. Sahebkar).

https://doi.org/10.1016/j.canlet.2019.01.045
Received 10 September 2018; Received in revised form 27 January 2019; Accepted 29 January 2019

and mucositis, which could be consequences of the slow release of the encapsulated drug [23,24]. In addition, employing passive targeting is not an effective strategy for anti-cancer drug delivery in hypovascular tissues such as prostate, pancreas and liver tumors [25–27]. Finally, compelling evidence suggests that the release of encapsulated agents from stealth liposomes in tumor interstitium and cellular uptake of the released drug are the main challenges of passive targeting [1,12]. Considering the aforementioned limitations, there is a growing interest in elaborating novel types of liposomes with improved characteristics in terms of controlled drug release and therapeutic efficacy, including pH/temperature/photo-sensitive liposomes, magnetic/ultrasound responsive liposomes and ligand-targeted liposomes [6]. The most widely used strategy is based on the use of specific ligands that exhibit a high affinity for tumor cells while having no or low affinity for normal cells [28]. Conjugation of specific ligands to liposomes enhances tumor accumulation and internalization of encapsulated drugs. Antibodies, antibody fragments, peptides, nucleic acids and small molecules are various ligands that have been employed for this purpose [29]. Among these ligands, aptamers are considered as a promising candidate with unique features for targeted delivery [30,31].

Aptamers are short, synthetic and single-stranded DNA or RNA molecules that can bind to their target with a high affinity and specificity [32]. Systematic evolution of ligands by exponential enrichment (SELEX) is a process which identifies aptamers from a random nucleic acid library [32]. Aptamers have several unique properties including small size, simple synthesis process, low immunogenicity, high affinity and target selectivity and stability across a wide range of physicochemical conditions. Furthermore, the production process of aptamers is performed out of the biological systems which reduces the risk of viral or bacterial contaminations [30]. Several properties of aptamers make them attractive therapeutic agents and potential alternatives to antibodies (Table 1). Some studies have shown that aptamers have privileged characteristic over antibodies especially in the field of oncology. Aptamers do not have the Fc region which stimulates the immune system in systemic administration. In addition, aptamers have higher tumor penetration, retention and homogenous distribution compared with antibodies [33,34]. Enhanced tumor penetration of aptamers is related to their smaller size. Previous studies suggested optimization of the size of antibodies to overcome poor penetration [35]. Furthermore, with smaller size, aptamers can be attached to the surface of nanoparticles with higher density without steric hindrance. The attachment process of aptamers to the surface of nanoparticles is more amenable and reproducible than antibodies [36,37]. Hence, replacement of antibodies by aptamers is a potential strategy to improve tumor penetration of nanoparticles. Owing to these features, aptamers are considered as promising ligands for active targeting purposes.

Despite the broad application of actively targeted nanoparticles in research, only a handful of them have advanced to clinical trial stages of development [38]. Currently, nanoparticles that are approved for clinical use generally lack active targeting and work on the basis of passive targeting. Clinical studies have shown that despite reduced cytotoxicity of nanoparticle-encapsulated chemotherapeutic drugs, the overall survival is not prolonged with such approaches compared to the free drug [39,40].

As witnessed by many studies, liposomes are by far the most successful type of nanoparticles used in drug delivery. They are well-investigated nanocarriers owing to their unique ability to entrap a diverse range of hydrophilic and hydrophobic drugs. Liposomes are also biocompatible delivery systems with the capacity of self-assembly. The characteristics of liposomes can be modified to achieve optimal biological properties without losing their stability or function [41]. Moreover, aptamers are promising targeting ligands with optimal specificity and selectivity. Here, we review the studies that have employed aptamers as targeting ligands in liposomal formulations and discuss the methods used for the preparation of aptamer-functionalized liposomes as well as the factors affecting the efficiency of aptamer coupling to liposomes.

2. Conjugation strategies in aptamer-targeted liposomes

Conjugation of aptamers to the surface of liposomes can be achieved through physical or chemical methods. Non-covalent attachment is based on the electrostatic coupling of negatively charged aptamers to positively charged liposomes. Such an electrostatic conjugation has been frequently used with cationic liposomes, particularly for the delivery of nucleic acid-based molecules in gene delivery experiments [42]. However, in many studies, even with cationic liposomes, covalent attachment is the preferred method of linking. Covalent linkage provides a better stability of the formulation in the biological environment and the detrimental effects of pH, temperature changes or presence of other biomolecules are minimized [42,43]. For covalent linkage, usually terminally modified aptamers are attached to the lipid chains or free terminus of polyethylene glycol (PEG) chains (Fig. 1). The following sections summarize the two main methods that are widely employed for covalent coupling of aptamers to the surface of liposomes, *i.e.* membrane anchor method and post-insertion method.

2.1. Membrane anchor method (pre-conjugation strategy)

In the membrane anchor method, aptamers are incorporated into liposome structure during the preparation process. Pre-conjugation is a one-step procedure that facilitates the purification of liposomal formulation [44]. The major drawback of pre-conjugation is the exposure of aptamers to organic solvents. The secondary structure of aptamers may be adversely affected by solvents used in the formulation procedure [44]. In this method, a portion of the aptamer molecules is located

Table 1
Properties of aptamers versus monoclonal antibodies ([118–121]).

	Monoclonal antibodies	Aptamers
Binding affinity	nanomolar to picomolar range	nanomolar to picomolar range
Development Process	Requires an immune response in animal model	SELEX (Systematic evolution of ligands by exponential enrichment) process
Manufacturing Process	*In vivo* production; Cell culture	*In vitro*, Chemical synthesis
Cost of production	Reasonably high and time consuming	Inexpensive and take a few weeks
Reproducibility	Little batch-to-batch variation	Activity of antibodies varies from batch to batch
Modification	Antibodies are typically conjugated with one type of signaling or binding molecule	Wide variety of chemical modifications are introduced to diversify properties and functions
Stability	susceptible to high temperatures and pH changes; irreversible denaturation	fairly stable at wide range of temperature; reversible denaturation
Immunogenicity	Significant Immunogenicity	No evidence of Immunogenicity
Structural diversity	Higher chemical diversity as the building blocks of antibodies are 20 different amino acids	Lower chemical diversity, as aptamers have only four building blocks (nucleotides). However, their diversity can be increased by chemical modification
Size	~150–170 kDa	~12–30 kDa
Target Size	≥600 Da	≥60 Da

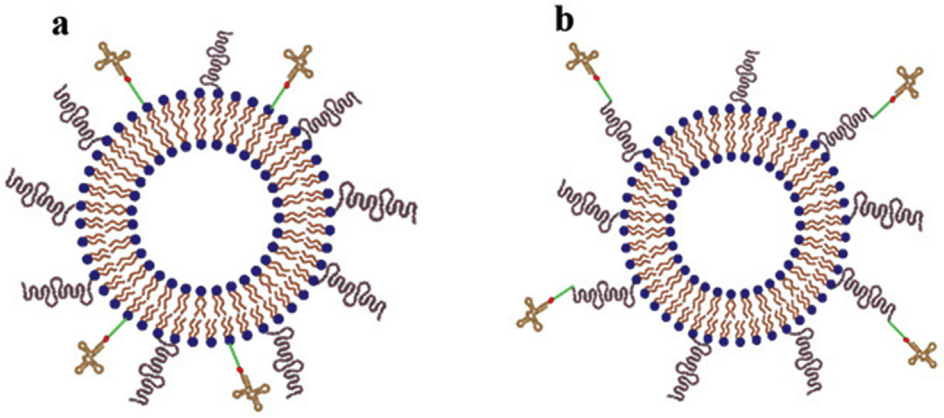

Fig. 1. Schematics of aptamer attachment to PEGylated-liposomes. aptamers conjugated directly on the phospholipid headgroups of PEGylated liposomes (a); and aptamers conjugated on the free terminus of PEGylated chains (b).

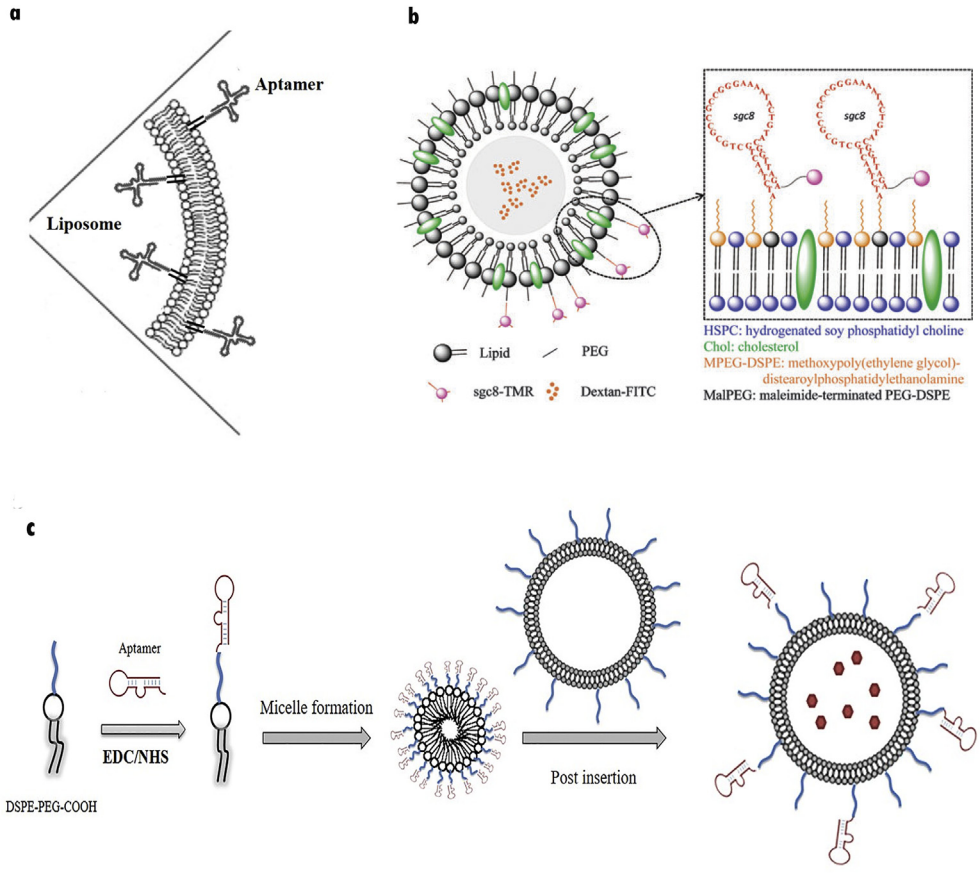

Fig. 2. Schematic representation of liposome-anchored aptamers. It is clearly represents a portion of aptamers are located in the inner surface of liposomes (a); Schematic illustration of the sgc8 aptamer anchoring in liposome structure. In this model the sgc8 aptamer covalently linked to the liposome by PEG spacer (b) [47]; Schematic showing the incorporation procedure of DSPE-PEG-aptamer micelles in liposomes structure by post-insertion method (c). Reproduced with permission (License code: 4366460801525).

in the inner surface of liposomes. It has been found that the presence of ligands or lipid-PEG ligands in the inner phase of liposomes could limit the internal space of liposomes and make liposomes susceptible to hydrolytic degradation [45]. Furthermore, internal aptamers could react with encapsulated active agents like doxorubicin. Willis et al. attached the NX213 aptamer to di(otadecyl)glycerol (DAG) and then DAG-NX213 was incorporated into liposome structures (Fig. 2a). This incorporation significantly increased plasma residence time of aptamers while the NX213 aptamers retained their inhibitory effect on VEGF-induced angiogenesis. However, about one-third of the aptamers were located inside the liposomes. Ribonuclease T1 digestion was used to determine what portion of aptamers was incorporated in the inner phase of liposomes [46]. Tan and colleagues conjugated 3′-thiol-modified sgc8 aptamer to maleimide-terminated PEG-DSPE (MalPEG) and constructed liposomes containing HSPC/cholesterol/mPEG-DSPE/MalPEG-DSPE) through extrusion method (Fig. 2b). Aptamer conjugation enhanced the interaction of liposomes with the target cells [47]. In two separate studies, liposomal formulations of doxorubicin and cisplatin were functionalized using AS1411 aptamers. Aptamers were attached to cholesterol and incorporated to liposomes using the membrane anchor method [48,49].

2.2. Post-insertion method

The second method used for the covalent coupling of aptamers to the surface of liposomes is post-insertion method (Fig. 2c). In this

method, functional group-activated lipid chains react with aptamers and the resulting unstable aptamer-modified lipid micelles are incubated with pre-formed drug-loaded liposomes [50]. Post-insertion is a simple method which provides efficient incorporation of targeting ligands into liposomal membrane. This method is flexible and compatible with a variety of ligands, thus allowing fabrication of different combinational constructs [51].

3. Factors affecting the efficiency of aptamer-functionalized liposomes

Different factors can affect the efficiency of aptamer-targeted liposomes. Some factors are related to the environment such as pH, temperature and biological enzymes. The effect of environmental factors could be minimized by proper formulation of aptamer-targeted liposomes. The following sections will discuss the parameters that must be considered in aptamer-targeted liposome formulations.

3.1. Conjugation chemistry of aptamers and liposomes

Bonding *via* amino acid group and sulfhydryl group are the most common linkage method that has been utilized for aptamer-lipid attachment. Thiol-maleimide reaction is widely used for aptamer bioconjugation [43]. In this process, the SH-modified aptamer is conjugated to the mPEG-DSPE-maleimide. Succinimidyl ester-amine reaction has also been used in many studies [43]. This process was exploited to attach aptamers to the surface of polymeric nanoparticles [52]. We have used this method for aptamer-liposome conjugation. In this process, carboxyl group of PEGylated-1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (PEG-DSPE-COOH) is first activated by N-hydroxysuccinimide (NHS) in the presence of ethyl(dimethylaminopropyl) carbodiimide (EDC). Then, activated carboxylate is incubated with amine-modified aptamer [53]. In some studies, carboxylate-modified aptamer has been used to bind to PEG-DSPE-NH_2 micelles. Both methods have appropriate binding efficiency and stability. Farokhzad et al. have addressed a potential problem of SH-modified aptamer, which is oxidation of thiol groups and disulfide bond created between aptamers [43]. Reducing agents such as tris (2-carboxyethyl) phosphine (TCEP) can prevent oxidation during the reaction process. They also mentioned that in succinimidyl ester-amine reaction, a small amount of unreacted carboxylate always remains on the surface of liposomes that reduces the zeta potential of liposomes. This phenomenon is supposed to be a potential advantage in cationic liposomes because it prevents non-specific reactions between negatively charged aptamers and liposome surface [43]. Another approach was proposed by Li et al. *who* attached the carboxyl group of anti-EGFR aptamer to the amine group of chitosan using the EDC/NHS technique; afterwards, the aptamer-chitosan conjugate was anchored into the liposome membrane [54].

3.2. The spacer structure

The attachment of aptamers to the surface of liposomes is usually performed *via* a spacer. A spacer located between aptamers and surfaces of liposomes is usually required to facilitate aptamer-lipid attachment and minimize interference from the liposome that might compromise the ligand recognition by aptamers. Different spacers have been exploited such as hydrocarbons, nucleotide tails or PEGs. Spacers are different in size and hydrophobicity. The length of spacer must be long enough to prevent liposome interference with aptamer binding. Hydrophilicity of spacers impacts on membrane diffusion of nanoparticles and their stability in blood circulation and should be carefully adjusted [55]. Kokkoli and coworkers studied how the varying properties of spacers affect the binding of the aptamer to the amphiphile moiety [56]. They showed nucleotide spacers allowed the highest efficiency in binding of aptamers to their target cells. PEG spacer was in the second place. Hydrocarbon chains and aptamer-lipid conjugate without spacer unit had the lowest cytotoxicity. It was also shown that the effect of spacer depends on the reaction medium because the presence of different ions or other substances may change the structure of aptamers. More recently, the effect of length and type of spacer on the targeting efficiency of aptamer-targeted liposomal doxorubicin was investigated. The results showed how the length of oligoT (thymine) spacer alters the interaction between AS1411 aptamer and backfilled PEG on the surface of liposomes [57]. Importantly, the obtained data demonstrated that to achieve the best targeting effect, aptamers should be fully exposed on the surface of liposomes and the shielding effect of PEG chains should be minimized [57]. Dai et al. also revealed that to evade shielding effect of PEG, the length of backfilled PEG on the surface of liposomes must be less than the length of the spacer [58]. As a strategy, PEG chains were recruited as the spacer and this significantly reduced the PEG interference [53,59,60]. Baek and coworkers used a tethering linker DNA to attach RNA aptamer to PEG chain. In this method, RNA aptamer was fully exposed on the surface of liposomes [61]. Spivak et al. showed that an increase in binding efficiency is due to the correct orientation of aptamers as aptamers are moved away from the surface of liposomes [62]. Spacers allow aptamers to be displayed on the surface of liposomes such that aptamers are accessible to their receptors. It seems reasonable to assume that the length of spacers, rather than their structure, is more critical to achieve the best targeting effect.

3.3. Aptamer characteristics

The function of aptamers is directly dependent on their structures. Folding into a unique structure, including stem and loop, has a pivotal role in target recognition of aptamers. The binding sites of aptamers can be determined by secondary structure prediction. Any disruption of the structure of aptamers lowers aptamer binding affinity [63]. Aptamer-target interactions are mediated by hydrophilic bindings that include hydrogen bonding as well as polar and electrostatic interactions. Some investigations attempted to use modified nucleotide libraries to provide additional hydrophobic interactions between aptamers and proteins [64–66].

A significant characteristic of highly specific aptamers is the presence of guanine-rich sequence in their structure. Aptamers that form guanine-quadruplex (G-quadruplex) in their structure have potential advantages for targeted delivery. G-quadruplex structures are highly resistant to serum nucleases and have higher thermal stability. Thus, they enhance stability and cellular uptake of aptamers. Moreover, aptamers containing G-quadrapelx have more affinity to their targets [67,68]. Methods of improving aptamers' affinity have been comprehensively reviewed by Hasegawa et al. [69].

Chemical modification of aptamers increases their stability and efficiency. Nuclease degradation is the first challenge that aptamers face *in vivo*. This challenge can be overcome by chemical modification of aptamer structure. Modification of natural nucleotides (*i.e.* 2′-OCH_3, 2′-NH_2 or 2′flouropyrimidine), substituting natural nucleotides with hydrocarbon linkers or capping terminal ends of aptamers are some chemical techniques that have been suggested [43]. Chemical stability of aptamers also limits the effect of environmental factors (temperature, pH and ionic strength) on the binding of aptamers to their targets [70].

How the length of aptamers influences their affinity is a controversial issue. The binding affinity of aptamers to their targets is strongly impacted by loop regions of the structure. After SELEX, the length of aptamers should be shortened by laborious strategies such as mapping or footprinting analysis. Keeping the loop sequence is necessary to save aptamer's binding ability [71,72]. Smaller aptamers may lose their affinity to target after direct attachment to the surface of nanoparticles. One explanation is that immobilized aptamers are not able to fold and create the second structure, thus they cannot bind to their target. Recruiting a spacer is highly recommended to help retaining aptamers' structure and properties. In addition, longer aptamers

can create more complex loop structures and possess more binding sites but, on the other hand, aptamer size may exert a considerable influence on the pharmacokinetic of the functionalized liposome. Surface functionalization alters the size and charge of liposomes, which in turn changes their pharmacokinetic characteristics. Although aptamers have a smaller size than proteins, the rigid chain in their structure makes their size similar to that of proteins [44,73]. In that regard, our *in vivo* experiments have shown the impact of the size of aptamers on the pharmacokinetic properties of liposomes [53,59]. A study on antibodies revealed that the size and affinity of antibodies alter the diffusion and penetration of nanoparticles to the tumor tissue [74]. This concept can also be applied to aptamers. However, it is generally accepted that there are many advantages to minimize the length of aptamers [75,76]. A direct benefit of minimizing the length of aptamers could be the lower cost of producing shorter aptamers. Surveying the literature to identify the effect of length on targeting efficiency of liposome-aptamer conjugates leads to a controversial conclusion, which indicates the role of length is likely interrelated with other parameters. Various studies have shown that trimming aptamers could enhance their binding affinity, reduce the risk of immunological reactions and extensively reduce the production cost of aptamers [76–80].

3.4. Surface density of aptamers

The number of attached aptamers changes the surface charge of liposomes. Gu et al. showed that the surface density of aptamers influences the tumor penetration of nanoparticles and should be optimized [81]. An excessive amount of aptamers may mask PEG chains on the surface of liposomes, reduce the surface charge and prevent efficient cellular uptake [73]. Utilizing multivalent aptamers instead of monovalent aptamers reduces the number of aptamers needed for targeted delivery and could improve the binding efficiency of aptamers [31].

4. Aptamer-mediated targeted delivery of liposomes

The ultimate aim of functionalization of liposomes by aptamers (or any targeting ligand in a general sense) is achieving a delivery system which (a) accumulates in tumor cells with high specificity and efficiency, (b) has no toxic effect on other organs, and (c) lacks adverse effects on the pharmacokinetic of liposomes.

In most of the studies, aptamer-functionalized liposomes have been shown to work perfectly in selective delivery *in vitro* (Fig. 3a and b). Aptamers effectively enhance the uptake of liposomes which is the consequence of receptor-mediated endocytosis. It is well understood that aptamer-targeted nanoparticles are internalized into the cell *via* receptor-mediated endocytosis. Briefly, binding of aptamers to their receptor triggers cell signaling cascades that culminate in engulfment and internalization of liposomes. Then, internalized liposomes fuse with early endosomes. Early endosomes differentiate into late endosomes, which can either fuse with the cell membrane or with lysosomes and form endolysosomes. In endolysosomes, hydrolytic enzymes and pH change lead to the release of drugs [52,82]. The mechanism of intracellular delivery of nanoparticles has been discussed in detail by Chou and co-workers [83]. Behzadi et al. also published a comprehensive review about the cellular uptake of nanoparticles and the effect of different parameters that control the nanoparticle-cell interactions [84].

The effect of aptamers on the biological fate of liposomes is a controversial issue. There is no direct correlation between *in vitro* and *in vivo* results. Similarly, Rudnick et al. reported that higher affinity to target does not necessarily improve tumor penetration of antibodies [74]. One possible explanation might be that higher affinity leads to tight binding of antibodies to their targets that would not allow deep penetration in the tumor tissue. This finding can be generalized to other targeting ligands like aptamers. Aptamer attachment changes size, surface charge and hydrophilicity of liposomes. Consequently, aptamer-targeted liposomes may have a different biodistribution compared with non-targeted liposomes [34]. A common problem in aptamer-liposome systems is their rapid elimination from systemic circulation because of their negative charge and corresponding rapid clearance. In 2011, Laurent and coworkers suggested that surrounding of actively targeted nanoparticles by serum proteins is an important factor that can explain the inconsistency of the *in vitro* and *in vivo* results. They assumed actively targeted nanoparticles are covered by protein corona upon their entrance to the blood circulation that leads to mistargeting [85]. Subsequently, further studies showed how corona formation reduces targeting capability of ligand-targeted nanoparticles [86–88]. Varnamkhasti et al. showed that at the presence of bovine serum albumin, the targeting efficacy of MUC1 aptamer-functionalized SN-38 is reduced. Their results indicated that there was no difference between the cytotoxicity of targeted and non-targeted SN-38 when they were treated

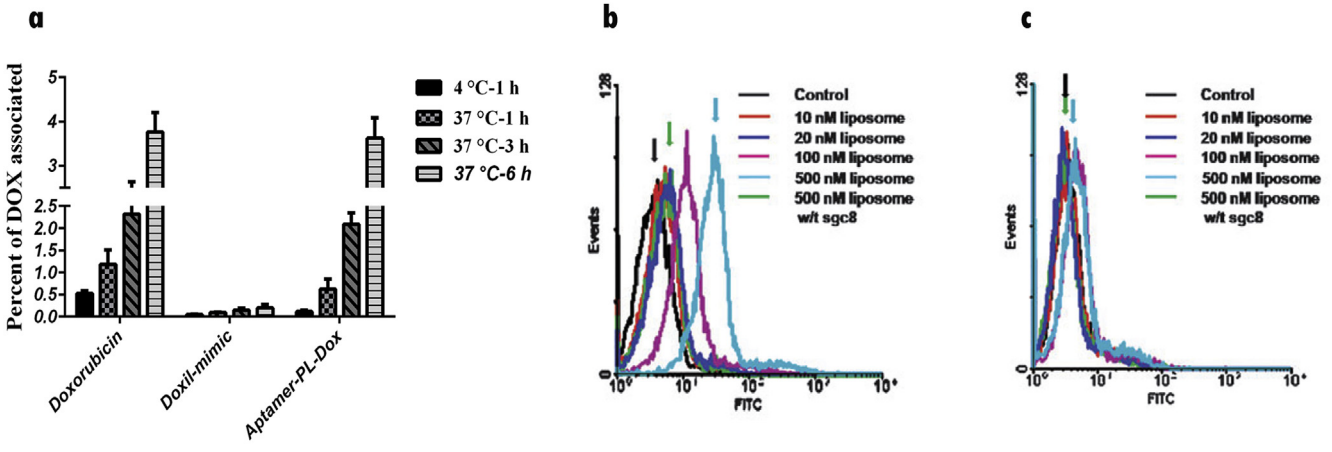

Fig. 3. The uptake of various aptamer functionalized liposomes by their target cells. Histogam of the alteration in cellular uptake of liposome at presence and absence of aptamers. Target cells were treated with different free drug, encapsulated drug in non-targeted liposomes and encapsulated drug in aptamer-targeted liposomes for 1, 3 and 6 h of incubation at either 37 °C or 4 °C. Then, the cells were lysed and percent of drug associated with cells was measured (a) [53]; Flow cytometry results indicate the uptake of various concentration of targeted liposomes in target cell line (b); compared with control cell line (c) after 30 min incubation with sgc8-targeted liposomes and non-targeted liposomes. The black = control cells without liposomes, the skyblue = 500 nM liposomes, and the green = 500 nM liposome without aptamer [47]. Reproduced with permission (License codes: 4366461502590 and 4366460801525). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

with bovine serum albumin before experiments [89]. To mimic physiological conditions, Ding and colleagues investigated the effect of human blood serum on targeting efficacy and release profile of aptamer-functionalized gold nanoparticles. They found that adsorption of proteins on the surface of aptamer-targeted nanoparticles, which could induce immune response, resulted in increased clearance rate of nanoparticles. They also showed that the effect of protein aggregation on the surface of nanoparticles is more critical in smaller nanoparticles [90].

In our studies, aptamer attachment increased the uptake of liposomes by liver and spleen [53,59]. Nevertheless, some studies reported an increase in the circulation time of liposomes after aptamer conjugation [91]. It is supposed that the negative charge of aptamer-liposome conjugates prevents the interaction of liposomes with negatively charged immune cells. The effect of the attached aptamer on the pharmacokinetic profile of liposomes could be minimized by employing a truncated version of aptamers. As mentioned before, it seems that the smaller aptamers have less effects on zeta potential and pharmacokinetic properties of liposomes [53,59]. Perschbacher and co-workers addressed how guanosine-rich structures impact on the pharmacokinetic profile of aptamers by affecting penetration through endothelial barriers and tissue uptake [92].

According to our findings, tumor accumulation was not affected by a decrease in half-life of aptamer-functionalized liposomes. In other words, the positive impact of aptamers on tumor accumulation of liposomes was more than the negative impact of them on half-life (Fig. 4a and b). Pharmacokinetic analysis showed that selective delivery of doxorubicin in aptamer-liposome conjugates was higher than non-targeted liposomes. Increased tumor accumulation and better cell internalization resulted in extended survival *in vivo* [53,59].

Findings by different groups suggest that aptamer functionalization enhances tumor accumulation of liposomes irrespective of any changes in pharmacokinetic parameters. Tumor growth is decelerated whether clearance is decreased or increased (Fig. 4c). It seems that the effect on tumor accumulation enhancement has a more important role in the efficacy of active targeting compared with clearance or half-life changes [42,48,53,59,61,91,93]. This apparent paradox could be explained by the mechanism of delivery of liposomes at the tissue and cellular level. At the tissue level, liposomes accumulate in the tumor by the EPR effect and targeting by aptamers does not facilitate this step. After tissue accumulation, targeted liposomes are able to enter the cells *via* ligand-mediated internalization. In other words, enhanced internalization leads to the release of the drug near to its site of action and increases antitumor efficacy of the drug [94]. Surveying *in vivo* experiments reveals that internalization of aptamer-targeted liposomes plays a critical role in improving the anti-tumor efficacy of the encapsulated drug [95]. It seems that one of the major pitfalls of aptamer-liposome conjugates relates to the fact that aptamers do not improve overall tumor penetration of liposomes. The poor tumor penetration can significantly reduce targeting efficiency of formulations. High tumor density, high interstitial fluid pressure and dense tumor matrix significantly reduce deep tumor penetration [1]. In animal models, solid tumors grow fast and most of the barriers are less effective; therefore, it is difficult to extrapolate animal data to humans for predicting the therapeutic efficacy of formulations [1,96]. Hence, finding a suitable model is necessary to evaluate the efficacy of these systems. Given the findings by various groups discussed above, it appears that despite alteration of the pharmacokinetic profile of liposomes and poor tumor accumulation or penetration, aptamer-targeted liposomes reduce tumor size of animal models more efficiently than non-targeted liposomes. As Storm et al. mentioned, active targeting of liposomes may increase their retention time in tumors and prevent them from rapid re-entry to the systemic circulation [1]. By this way, aptamer-targeted liposomes remain longer in the tumor space leading to enhanced anti-tumor efficacy compared with non-targeted liposomes.

In the last few decades, many aptamers have been developed to recognize cancer-specific biomarkers. Some developed aptamers against cancer biomarkers are summarized in Table 2. In the following sections, we review aptamer-functionalized liposomal systems with an emphasis on the receptors targeted on the surface of cancer cells or related tissues.

4.1. Protein tyrosine kinase 7 (PTK7)

Protein tyrosine kinase 7 (PTK7) is a transmembrane receptor highly expressed on human acute lymphoblastic leukemia cells (CEM). Tan et al. utilized scg8 aptamer for the delivery of liposomes loaded with fluorescein-dextran as a drug mimic. 3′-thiol-modified sgc8 aptamer was attached to maleimide-functionalized lipid chain. The study showed that attachment of scg8 aptamer to the surface of liposomes significantly increased the uptake of liposomes into CEM cells [47].

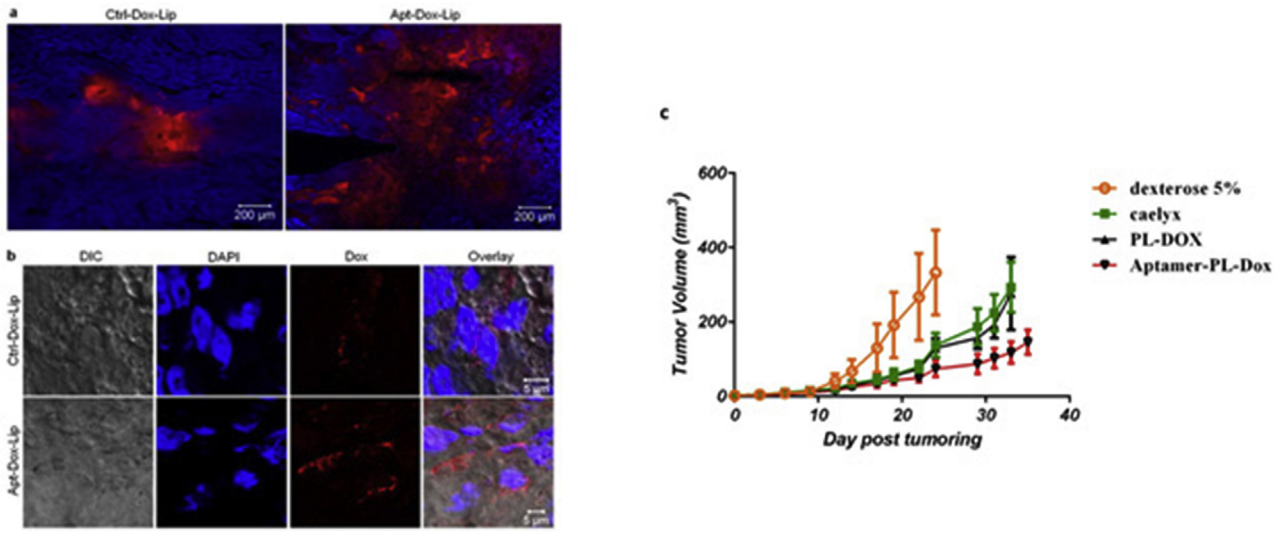

Fig. 4. In vivo tumor penetration study. Fluorescence microscope images of MCF-7 tumor sections shows tumor penetration of liposomal Doxorubicin decorated by AS1411 aptamer or non-aptameric DNA sequence (a&b) [48]; The graph shows the tumor growth rate after intravenous injection of aptamer-targeted liposomes in balb/c mice in comparison with non-targeted liposomes and control group (c) [53]. Reproduced with permission (License codes: 4366460472886 and 4366461502590).

Table 2
Aptamers targeting cancer biomarkers.

Aptamer/type	Nucleotide number	target	Therapeutic application	reference
AS1411/DNA	26	Nucleolin	Induce bcl-2 mRNA instability	[104, 122]
5TR1/DNA	25	Mucin-1	Prevent cancer cell invasion through beta catenin	[123]
S2.1/DNA	25			
EpCAM/RNA	18	EpCAM	Regulate gene expression of c-myc, e-fabp, cyclin, and modulate EMT	[124]
xPSMA9/RNA	40	PSMA (prostate specific membrane antigen)	Prevent hydrolysis of N-acetylaspartyl-glutamate for over-proliferation	[61, 101]
A10/RNA	40			
TA1/DNA	30	CD44	Inhibit cell proliferation, differentiation, migration, and angiogenesis	[100, 125]
Apt1/RNA	90			
Anti-EGFR/DNA	30	EGFR (Epidermal growth factor receptor)	Inhibit cell proliferation, invasion and metastasis	[54, 111]
HB5/DNA	86	HER2 (human epidermal growth factor 2)	Inhibition of tumorigenic signaling	[126, 127]
Apt/DNA	31			
A30/RNA	49	HER3 (human epidermal growth factor receptor 3)	Reduction of drug resistance	[128]

4.2. E-selectin

E-selectin is a protein expressed by inflamed endothelial cells and plays an important role in the adhesion of tumor cells to the vascular endothelium [97]. Mann et al. linked E-selectin-specific thiolated aptamer (ESTA) to fluorescein-encapsulated liposomes. *In vivo* studies have revealed that the tumor accumulation in the group receiving targeted liposomes was higher than that in the non-targeted liposome group. Pharmacokinetic analysis showed that both groups had the same volume of distribution (Vd), while aptamer-functionalized liposomes had slower clearance rate and slightly extended circulation half-time [91].

4.3. CD44 protein

CD44 receptor protein is overexpressed by many tumors and is one of the most common cancer stem cell surface markers. It plays an important role in the invasion of tumor cells which makes it an attractive receptor for therapeutic targeting [98]. Alshaer et al. designed CD44 RNA aptamer (apt1) and attached it to the surface of liposome. They showed aptamer-conjugated liposomes had higher selectivity to $CD44^{+}$ cell line compared with non-targeted liposomes. Apt1 was attached to the surface of liposomes *via* thiol-maleimide reaction [98,99].

4.4. Prostate-specific membrane antigen

Prostate-specific membrane antigen (PSMA) is a well-characterized membrane protein that is overexpressed on the surface of prostate cancer cells [31]. PSMA is widely used as a target of drug delivery systems. Lupold et al. selected A9 and A10 RNA aptamers using *in vitro* SELEX against PSMA [100]. xPSM-A9 is a PSMA-specific RNA aptamer that was used for targeted delivery of liposomes by Baek et al. xPSM-A9 was attached to the surface of liposomes containing rhodamine or doxorubicin. The results revealed that xPSM-A9 significantly increased cellular uptake of liposomes in a PSMA-positive cell line. The *in vivo* experiments showed that aptamer functionalization increases tumor accumulation of liposomes and decelerates tumor growth over time compared with non-targeted liposomes [61]. PSMA-specific aptamer-liposome conjugates have also been proposed for the delivery of radioactive actinium (225Ac) in antivascular radiotherapy [101]. Stuart et al. reported the targeting of zinc chelator-laden liposomes by SZTI01 aptamer. SZTI01 targets PSMA. Zinc chelator N,N,N′,N′-tetrakis (2-pyridylmethyl)-ethylenediamine (TPEN) action leads to unbalancing oxygen species, thereby inducing cell death. The results revealed that aptamer targeting reduced tumor growth in mice with human prostate cancer xenograft [93].

4.5. Nucleolin protein

Nucleolin is a multifunctional protein which is overexpressed in many types of tumor cells [102]. Nucleolin plays a central role in angiogenesis in tumor cells and inhibits apoptosis. The most advanced aptamer in target delivery and cancer treatment is the nucleolin-specific aptamer AS1411. AS1411 is a 26-mer G-rich DNA aptamer which inhibits cell proliferation in a wide range of cancers [103,104]. AS1411 is the first anti-cancer aptamer that had reached clinical stage evaluation [103]. Lu and coworkers in 2009 incorporated AS1411-cholesterol into liposome membrane to enhance the delivery of cisplatin. AS1411-liposomes showed higher cytotoxicity compared to non-targeted liposomes. They Authors also treated nucleolin-expressing MCF7 cells with

liposomes containing calcein as a fluorescent dye with and without AS1411 targeting. MCF7 cells treated with AS1411-liposomes showed a strong fluorescent signal compared to non-targeted liposomes or non-expressing AS1411 cells [49]. They further explored the impact of AS1411 aptamer in target delivery of liposomal doxorubicin. The results showed that AS1411 significantly increased cell internalization. In addition, antitumor efficiency of liposomes was improved in the mouse model [48]. Sung et al. designed thermosensitive liposomes functionalized with AS1411 aptamer to overcome slow drug release from liposomes. AS1411 was attached to the surface of liposomes containing doxorubicin and ammonium bicarbonate as a thermoresponsive trigger. It was found that cytotoxicity was increased after treatment of cells with AS1411-coupled liposomes followed by hyperthermia. Moreover, a better result was achieved in mice at 42 °C compared to 37 °C [105]. Du et al. also employed AS1411 aptamer for targeted delivery of thermosensitive docetaxel-loaded liposomes. They used dual aptamer targeting by AS1411 and S2.2 aptamers against nucleolin and MUC1 receptors. They also coated the surface of liposomes with gold nanoshells. Their results exhibited higher tumor accumulation while in other organs concentration of docetaxel was reduced [106]. Similarly, the use of AS1411-coupled thermosensitive liposomes for magnetic resonance imaging in cancer diagnosis was clearly demonstrated [107]. Yung and coworkers employed an AS1411 aptamer-liposome conjugate for the delivery of anti-BRAF siRNA (siBraf) for the treatment of melanoma [42]. They reported strong silencing of BRAF gene and inhibition of melanoma growth in xenografted mice.

4.6. Transferrin receptor

The transferrin receptor is another receptor that is upregulated on the surface of many cancer cells and is widely studied in targeted delivery to the tumor tissue [108]. Wilner and coworkers designed RNA aptamers against transferrin and used it for targeted delivery of siRNA-containing liposomes. 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane (DLinDMA) was used as a positively charged lipid in the formulation. SH-modified aptamer was attached to DSPE-PEG-maleimide *via* thiol-maleimide conjugation. The results showed that aptamer targeting improved cellular uptake of liposomes and improved gene knockdown efficiency [109].

4.7. Epidermal growth factor receptor (EGFR)

Epidermal growth factor receptor (EGFR) is overexpressed in many solid cancers and the activation of EGFR is associated with cell proliferation, invasion, metastasis, angiogenesis and decreased apoptosis in malignant cancers [110]. Li and coworkers appended anti-EGFR aptamer on the surface of liposomes containing erlotinib *via* aptamer-chitosan anchoring. Aptamer conjugation enhanced cytotoxicity of liposomes compared with non-targeted counterparts in a lung cancer cell line [54].

4.8. Others

In some studies, aptamers obtained from whole cell-based SELEX process were used for targeted delivery. In cell-based SELEX, aptamers interact with cell surface receptors in living cells. Sometimes the aim of cell-SELEX is to obtain an aptamer against highly expressed receptors. However, in other cases aptamers are selected against a specific cell line with no particular surface receptor in mind [31]. Harashima and colleagues reported AraHH001 DNA aptamer-modified PEGylated liposomal rhodamine for targeted delivery toward tumor vasculature. Aptamer-modified liposomes showed a high selectivity for tumor vascular cells without binding to healthy cells *in vitro*. The mentioned liposomes also showed increased tumor vasculature accumulation *in vivo* [111].

Liu et al. exploited IL-4Rα aptamer-liposome to overcome tumor microenvironment and deliver CpG to tumor cells [112]. As expected, targeting liposomes by IL-4Rα aptamer improved cellular uptake of liposomes and distribution of liposomes in the tumor tissue. In mice bearing CT26 tumor, IL-4Rα aptamer enhanced anti-tumor efficiency of liposome-CpG and reduced tumor growth.

5. Future perspectives and conclusion

SELEX method was developed 28 years ago for isolating aptamers as targeting agents [32,113]. Since then, aptamers have gained increasing utility in active targeting of nanoparticles. The first FDA-approved aptamer is pegaptanib (Macugen®) that is a 27-nucleotide RNA aptamer. Pegaptanib is used to treat age-related macular degeneration [114]. Some therapeutic aptamers for hemostasis and diabetes mellitus have also entered the clinical trial phase. AS1411 and Sgc8 aptamers are undergoing clinical trials for cancer treatment [115,116]. These aptamers have been described in previous sections. As mentioned before, AS1411 and Scg8 are short-length aptamers and may have less interference with the surface characteristics of nanoparticles.

The high manufacturing cost of aptamers is a notable limitation in the development of aptamer-functionalized liposomes. Another issue is that monoclonal antibodies have a well-established setup technology in many laboratories; thus, many researchers prefer to work on antibodies. However, aptamer synthesis technology is improving and patents related to aptamers will expire in the following years [73]. Thus, it could be expected that manufacturing costs fall and many companies invest in aptamer technology.

We expect that future efforts in the field of aptamer-liposome conjugates will be centered on choosing suitable aptamers as targeting ligands. In addition, optimization of aptamers by either advanced SELEX selection methods against targeting agents or new screening techniques can improve the target efficiency of aptamers. In parallel to the development of new aptamers, the effect of tumor microenvironment should be carefully probed. Most of the *in vivo* experiments have used single dose regimens and it could be assumed that most of the injected aptamer-liposomes cannot reach their site of action and penetrate tumor tissue efficiently. In multiple-dose regimens, larger doses may penetrate farther in tumor tissue. This is because in multiple-dose administration, there is a continuous efflux from the surrounding tissue into tumor [117]. We suggest that using a multiple-dose regimen may overcome the high-density barrier of the tumor and enhance tumor penetration.

In spite of the challenges, the potential of aptamer-targeted liposomes as cancer-targeted drug delivery systems could lead to the design of targeted delivery systems with more predictable clinical behavior.

Conflicts of interest

None.

Source of funding

This project has been conduct by a grant from cancer research center of cancer institute of Iran (Sohrabi cancer charity, Grant No: 37652-202-01-97). This project was also financially supported by grant No. 961204 of the Biotechnology Development Council of the Islamic Republic of Iran".

References

[1] T. Lammers, F. Kiessling, W.E. Hennink, G. Storm, Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress, J. Contr. Release : Off. J. Contr. Release Soc. 161 (2012) 175–187.
[2] R.K. Jain, T. Stylianopoulos, Delivering nanomedicine to solid tumors, Nat. Rev. Clin. Oncol. 7 (2010) 653–664.
[3] J. Shi, P.W. Kantoff, R. Wooster, O.C. Farokhzad, Cancer nanomedicine: progress, challenges and opportunities, Nat. Rev. Canc. 17 (2017) 20–37.
[4] Y. Malam, M. Loizidou, A.M. Seifalian, Liposomes and nanoparticles: nanosized

vehicles for drug delivery in cancer, Trends Pharmacol. Sci. 30 (2009) 592–599.
[5] T.M. Allen, D.R. Mumbengegwi, G.J.R. Charrois, Anti-CD19-Targeted liposomal doxorubicin improves the therapeutic efficacy in murine B-cell lymphoma and ameliorates the toxicity of liposomes with varying drug release rates, Clin. Cancer Res. 11 (2005) 3567–3573.
[6] T.M. Allen, P.R. Cullis, Liposomal drug delivery systems: from concept to clinical applications, Adv. Drug Deliv. Rev. 65 (2013) 36–48.
[7] Y.P. Patil, S. Jadhav, Novel methods for liposome preparation, Chem. Phys. Lipids 177 (2014) 8–18.
[8] Y. Barenholz, Doxil(R)–the first FDA-approved nano-drug: lessons learned, J. Contr. Release : Off. J. Contr. Release Soc. 160 (2012) 117–134.
[9] A. Gabizon, H. Shmeeda, Y. Barenholz, Pharmacokinetics of pegylated liposomal Doxorubicin: review of animal and human studies, Clin. Pharmacokinet. 42 (2003) 419–436.
[10] S. Fathi, A.K. Oyelere, Liposomal drug delivery systems for targeted cancer therapy: is active targeting the best choice? Future Med. Chem. 8 (17) (2016 Nov) 2091–2112.
[11] H. Maeda, Y. Matsumura, Tumoritropic and lymphotropic principles of macromolecular drugs, Crit. Rev. Ther. Drug Carrier Syst. 6 (1989) 193–210.
[12] N. Bertrand, J. Wu, X. Xu, N. Kamaly, O.C. Farokhzad, Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology, Adv. Drug Deliv. Rev. 66 (2014) 2–25.
[13] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review, J. Contr. Release : Off. J. Contr. Release Soc. 65 (2000) 271–284.
[14] H. Maeda, T. Sawa, T. Konno, Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS, J. Contr. Release : Off. J. Contr. Release Soc. 74 (2001) 47–61.
[15] B.S. Pattni, V.V. Chupin, V.P. Torchilin, New developments in liposomal drug delivery, Chem. Rev. 115 (2015) 10938–10966.
[16] J.A. Ellerhorst, A. Bedikian, S. Ring, A.C. Buzaid, O. Eton, S.S. Legha, Phase II trial of doxil for patients with metastatic melanoma refractory to frontline therapy, Oncol. Rep. 6 (1999) 1097–1099.
[17] A.A. Garcia, R.A. Kempf, M. Rogers, F.M. Muggia, A phase II study of Doxil (liposomal doxorubicin): lack of activity in poor prognosis soft tissue sarcomas, Ann. Oncol. : Off. J. Europ. Soc. Med. Oncol. 9 (1998) 1131–1133.
[18] S. Halford, D. Yip, C.S. Karapetis, A.H. Strickland, A. Steger, H.T. Khawaja, P.G. Harper, A phase II study evaluating the tolerability and efficacy of CAELYX (liposomal doxorubicin, Doxil) in the treatment of unresectable pancreatic carcinoma, Ann. Oncol. : Off. J. Europ. Soc. Med. Oncol./ESMO 12 (2001) 1399–1402.
[19] Y. Matsumura, M. Gotoh, K. Muro, Y. Yamada, K. Shirao, Y. Shimada, M. Okuwa, S. Matsumoto, Y. Miyata, H. Ohkura, K. Chin, S. Baba, T. Yamao, A. Kannami, Y. Takamatsu, K. Ito, K. Takahashi, Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer, Ann. Oncol. : Off. J. Europ. Soc. Med. Oncol. 15 (2004) 517–525.
[20] T.L. Andresen, S.S. Jensen, K. Jorgensen, Advanced strategies in liposomal cancer therapy: problems and prospects of active and tumor specific drug release, Prog. Lipid Res. 44 (2005) 68–97.
[21] S. Bandak, D. Goren, A. Horowitz, D. Tzemach, A. Gabizon, Pharmacological studies of cisplatin encapsulated in long-circulating liposomes in mouse tumor models, Anti Canccer Drugs 10 (1999) 911–920.
[22] Y.L. Tseng, J.J. Liu, R.L. Hong, Translocation of liposomes into cancer cells by cell-penetrating peptides penetratin and tat: a kinetic and efficacy study, Mol. Pharmacol. 62 (2002) 864–872.
[23] L. Serpe, M. Guido, R. Canaparo, E. Muntoni, R. Cavalli, P. Panzanelli, C.D. Pepa, A. Bargoni, A. Mauro, M.R. Gasco, M. Eandi, G.P. Zara, Intracellular accumulation and cytotoxicity of doxorubicin with different pharmaceutical formulations in human cancer cell lines, J. Nanosci. Nanotechnol. 6 (2006) 3062–3069.
[24] G.J. Charrois, T.M. Allen, Multiple injections of pegylated liposomal Doxorubicin: pharmacokinetics and therapeutic activity, J. Pharmacol. Exp. Therapeut. 306 (2003) 1058–1067.
[25] E. Hagtvet, T.J. Evjen, D.R. Olsen, S.L. Fossheim, E.A. Nilssen, Ultrasound enhanced antitumor activity of liposomal doxorubicin in mice, J. Drug Target. 19 (2011) 701–708.
[26] C. Bode, L. Trojan, C. Weiss, B. Kraenzlin, U. Michaelis, M. Teifel, P. Alken, M.S. Michel, Paclitaxel encapsulated in cationic liposomes: a new option for neovascular targeting for the treatment of prostate cancer, Oncol. Rep. 22 (2009) 321–326.
[27] F. Danhier, O. Feron, V. Preat, To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery, J. Contr. Release : Off. J. Contr. Release Soc. 148 (2010) 135–146.
[28] P. Sapra, T.M. Allen, Ligand-targeted liposomal anticancer drugs, Prog. Lipid Res. 42 (2003) 439–462.
[29] V.P. Torchilin, Passive and active drug targeting: drug delivery to tumors as an example, Handb. Exp. Pharmacol. (2010) 3–53.
[30] G. Zhou, G. Wilson, L. Hebbard, W. Duan, C. Liddle, J. George, L. Qiao, Aptamers: a promising chemical antibody for cancer therapy, Oncotarget 7 (2016) 13446–13463.
[31] A.S. Barbas, J. Mi, B.M. Clary, R.R. White, Aptamer applications for targeted cancer therapy, Future Oncol. 6 (2010) 1117–1126.
[32] A.D. Ellington, J.W. Szostak, In vitro selection of RNA molecules that bind specific ligands, Nature 346 (1990) 818–822.
[33] D. Xiang, C. Zheng, S.-F. Zhou, S. Qiao, P.H.-L. Tran, C. Pu, Y. Li, L. Kong, A.Z. Kouzani, J. Lin, K. Liu, L. Li, S. Shigdar, W. Duan, Superior performance of aptamer in tumor penetration over antibody: implication of aptamer-based theranostics in solid tumors, Theranostics 5 (2015) 1083–1097.
[34] Y.H. Lao, K.K. Phua, K.W. Leong, Aptamer nanomedicine for cancer therapeutics: barriers and potential for translation, ACS Nano 9 (2015) 2235–2254.
[35] A.P. Chapman, PEGylated antibodies and antibody fragments for improved therapy: a review, Adv. Drug Deliv. Rev. 54 (2002) 531–545.
[36] A.V. Lakhin, V.Z. Tarantul, L.V. Gening, Aptamers: problems, solutions and prospects, Acta naturae 5 (2013) 34–43.
[37] S.D. Jayasena, Aptamers: an emerging class of molecules that rival antibodies in diagnostics, Clin. Chem. 45 (1999) 1628.
[38] A.C. Anselmo, S. Mitragotri, Nanoparticles in the clinic, Bioeng. Transl. Med. 1 (2016) 10–29.
[39] Z. Cheng, A.A. Zaki, J.Z. Hui, V.R. Muzykantov, A. Tsourkas, Multifunctional Nanoparticles: cost versus benefit of adding targeting and imaging capabilities, Science (New York, N.Y.) 338 (2012) 903–910.
[40] R.K. Jain, T. Stylianopoulos, Delivering nanomedicine to solid tumors, Nat. Rev. Clin. Oncol. 7 (2010) 653–664.
[41] L. Sercombe, T. Veerati, F. Moheimani, S.Y. Wu, A.K. Sood, S. Hua, Advances and challenges of liposome assisted drug delivery, Front. Pharmacol. 6 (2015) 286-286.
[42] L. Li, J. Hou, X. Liu, Y. Guo, Y. Wu, L. Zhang, Z. Yang, Nucleolin-targeting liposomes guided by aptamer AS1411 for the delivery of siRNA for the treatment of malignant melanomas, Biomaterials 35 (2014) 3840–3850.
[43] O.C. Farokhzad, J.M. Karp, R. Langer, Nanoparticle-aptamer bioconjugates for cancer targeting, Expert Opin. Drug Deliv. 3 (2006) 311–324.
[44] N. Bertrand, J. Wu, X. Xu, N. Kamaly, O.C. Farokhzad, Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology, Adv. Drug Deliv. Rev. 66 (2014) 2–25.
[45] O.K. Nag, V. Awasthi, Surface engineering of liposomes for stealth behavior, Pharmaceutics 5 (2013) 542–569.
[46] M.C. Willis, B.D. Collins, T. Zhang, L.S. Green, D.P. Sebesta, C. Bell, E. Kellogg, S.C. Gill, A. Magallanez, S. Knauer, R.A. Bendele, P.S. Gill, N. Janjic, Liposome-anchored vascular endothelial growth factor aptamers, Bioconjug. Chem. 9 (1998) 573–582.
[47] H. Kang, M.B. O'Donoghue, H. Liu, W. Tan, A liposome-based nanostructure for aptamer directed delivery, Chem. Commun. (Camb) 46 (2010) 249–251.
[48] H. Xing, L. Tang, X. Yang, K. Hwang, W. Wang, Q. Yin, N.Y. Wong, L.W. Dobrucki, N. Yasui, J.A. Katzenellenbogen, W.G. Helferich, J. Cheng, Y. Lu, Selective delivery of an anticancer drug with aptamer-functionalized liposomes to breast cancer cells in vitro and in vivo, J. Mater. Chem. B 1 (2013) 5288–5297.
[49] Z. Cao, R. Tong, A. Mishra, W. Xu, G.C. Wong, J. Cheng, Y. Lu, Reversible cell-specific drug delivery with aptamer-functionalized liposomes, Angew. Chem. 48 (2009) 6494–6498.
[50] V. Torchilin, Antibody-modified liposomes for cancer chemotherapy, Expert Opin. Drug Deliv. 5 (2008) 1003–1025.
[51] T.M. Allen, P. Sapra, E. Moase, Use of the post-insertion method for the formation of ligand-coupled liposomes, Cell. Mol. Biol. Lett. 7 (2002) 889–894.
[52] S. Dhar, F.X. Gu, R. Langer, O.C. Farokhzad, S.J. Lippard, Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt(IV) prodrug-PLGA-PEG nanoparticles, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 17356–17361.
[53] S.A. Moosavian, K. Abnous, A. Badiee, M.R. Jaafari, Improvement in the drug delivery and anti-tumor efficacy of PEGylated liposomal doxorubicin by targeting RNA aptamers in mice bearing breast tumor model, Colloids Surfaces B Biointerfaces 139 (2016) 228–236.
[54] F. Li, H. Mei, X. Xie, H. Zhang, J. Liu, T. Lv, H. Nie, Y. Gao, L. Jia, Aptamer-conjugated chitosan-anchored liposomal complexes for targeted delivery of erlotinib to EGFR-mutated lung cancer cells, AAPS J. 19 (2017) 814–826.
[55] M. Srinivasarao, P.S. Low, Ligand-targeted drug delivery, Chem. Rev. 117 (2017) 12133–12164.
[56] B. Waybrant, T.R. Pearce, E. Kokkoli, Effect of polyethylene glycol, alkyl, and oligonucleotide spacers on the binding, secondary structure, and self-assembly of fractalkine binding FKN-S2 aptamer-amphiphiles, Langmuir : ACS J. Surf. Coll. 30 (2014) 7465–7474.
[57] H. Xing, J. Li, W. Xu, K. Hwang, P. Wu, Q. Yin, Z. Li, J. Cheng, Y. Lu, The effects of spacer length and composition on aptamer-mediated cell-specific targeting with nanoscale PEGylated liposomal doxorubicin, Chembiochem : Europ. J. Chem. Biol. 17 (2016) 1111–1117.
[58] Q. Dai, C. Walkey, W.C. Chan, Polyethylene glycol backfilling mitigates the negative impact of the protein corona on nanoparticle cell targeting, Angew. Chem. 53 (2014) 5093–5096.
[59] S.A. Moosavian, K. Abnous, J. Akhtari, L. Arabi, A. Gholamzade Dewin, M. Jafari, 5TR1 aptamer-PEGylated liposomal doxorubicin enhances cellular uptake and suppresses tumour growth by targeting MUC1 on the surface of cancer cells, Artificial cells, Nanomedicine, and Biotechnology, 2017, pp. 1–12.
[60] J. Ma, H. Zhuang, Z. Zhuang, Y. Lu, R. Xia, L. Gan, Y. Wu, Development of docetaxel liposome surface modified with CD133 aptamers for lung cancer targeting, Artificial Cells, Nanomedicine, and Biotechnology 46 (2018) 1864–1871.
[61] S.E. Baek, K.H. Lee, Y.S. Park, D.K. Oh, S. Oh, K.S. Kim, D.E. Kim, RNA aptamer-conjugated liposome as an efficient anticancer drug delivery vehicle targeting cancer cells in vivo, J. Contr. Release : Off. J. Contr. Release Soc. 196 (2014) 234–242.
[62] S. Balamurugan, A. Obubuafo, R.L. McCarley, S.A. Soper, D.A. Spivak, Effect of linker structure on surface density of aptamer monolayers and their corresponding protein binding efficiency, Anal. Chem. 80 (2008) 9630–9634.
[63] A. Avino, C. Fabrega, M. Tintore, R. Eritja, Thrombin binding aptamer, more than a simple aptamer: chemically modified derivatives and biomedical applications,

Curr. Pharmaceut. Des. 18 (2012) 2036–2047.
[64] J.D. Vaught, C. Bock, J. Carter, T. Fitzwater, M. Otis, D. Schneider, J. Rolando, S. Waugh, S.K. Wilcox, B.E. Eaton, Expanding the chemistry of DNA for in vitro selection, J. Am. Chem. Soc. 132 (2010) 4141–4151.
[65] L. Gold, D. Ayers, J. Bertino, C. Bock, A. Bock, E.N. Brody, J. Carter, A.B. Dalby, B.E. Eaton, T. Fitzwater, D. Flather, A. Forbes, T. Foreman, C. Fowler, B. Gawande, M. Goss, M. Gunn, S. Gupta, D. Halladay, J. Heil, J. Heilig, B. Hicke, G. Husar, N. Janjic, T. Jarvis, S. Jennings, E. Katilius, T.R. Keeney, N. Kim, T.H. Koch, S. Kraemer, L. Kroiss, N. Le, D. Levine, W. Lindsey, B. Lollo, W. Mayfield, M. Mehan, R. Mehler, S.K. Nelson, M. Nelson, D. Nieuwlandt, M. Nikrad, U. Ochsner, R.M. Ostroff, M. Otis, T. Parker, S. Pietrasiewicz, D.I. Resnicow, J. Rohloff, G. Sanders, S. Sattin, D. Schneider, B. Singer, M. Stanton, A. Sterkel, A. Stewart, S. Stratford, J.D. Vaught, M. Vrkljan, J.J. Walker, M. Watrobka, S. Waugh, A. Weiss, S.K. Wilcox, A. Wolfson, S.K. Wolk, C. Zhang, D. Zichi, Aptamer-based multiplexed proteomic technology for biomarker discovery, PLoS One 5 (2010) e15004.
[66] M. Kimoto, R. Yamashige, K. Matsunaga, S. Yokoyama, I. Hirao, Generation of high-affinity DNA aptamers using an expanded genetic alphabet, Nat. Biotechnol. 31 (2013) 453–457.
[67] V. Viglasky, T. Hianik, Potential uses of G-quadruplex-forming aptamers, Gen. Physiol. Biophys. 32 (2013) 149–172.
[68] W.O. Tucker, K.T. Shum, J.A. Tanner, G-quadruplex DNA aptamers and their ligands: structure, function and application, Curr. Pharmaceut. Des. 18 (2012) 2014–2026.
[69] H. Hasegawa, N. Savory, K. Abe, K. Ikebukuro, Methods for improving aptamer binding affinity, Molecules 21 (2016) 421.
[70] X. Li, Q. Zhao, L. Qiu, Smart ligand: aptamer-mediated targeted delivery of chemotherapeutic drugs and siRNA for cancer therapy, J. Contr. Release 171 (2013) 152–162.
[71] S.C.B. Gopinath, T. Lakshmipriya, M.K. Md Arshad, C.H. Voon, T. Adam, U. Hashim, H. Singh, S.V. Chinni, Shortening full-length aptamer by crawling base deletion – assisted by Mfold web server application, Journal of the Association of Arab Universities for Basic and Applied Sciences, vol. 23, 2017, pp. 37–42.
[72] R.E. Armstrong, G.F. Strouse, Rationally manipulating aptamer binding affinities in a stem-loop molecular beacon, Bioconjug. Chem. 25 (2014) 1769–1776.
[73] Z. Xiao, O.C. Farokhzad, Aptamer-functionalized nanoparticles for medical applications: challenges and opportunities, ACS Nano 6 (2012) 3670–3676.
[74] S.I. Rudnick, J. Lou, C.C. Shaller, Y. Tang, A.J.P. Klein-Szanto, L.M. Weiner, J.D. Marks, G.P. Adams, Influence of affinity and antigen internalization on the uptake and penetration of anti-HER2 antibodies in solid tumors, Cancer Res. 71 (2011) 2250–2259.
[75] J.G. Bruno, A review of therapeutic aptamer conjugates with emphasis on new approaches, Pharmaceuticals 6 (2013) 340–357.
[76] T.T. Le, O. Chumphukam, A.E.G. Cass, Determination of minimal sequence for binding of an aptamer. A comparison of truncation and hybridization inhibition methods, RSC Adv. 4 (2014) 47227–47233.
[77] H. Kaur, L.Y. Yung, Probing high affinity sequences of DNA aptamer against VEGF165, PLoS One 7 (2012) e31196.
[78] A.D. Keefe, S. Pai, A. Ellington, Aptamers as therapeutics, Nature reviews. Drug Discovery, vol. 9, 2010, pp. 537–550.
[79] H. Mei, T. Bing, X. Yang, C. Qi, T. Chang, X. Liu, Z. Cao, D. Shangguan, Functional-group specific aptamers indirectly recognizing compounds with alkyl amino group, Anal. Chem. 84 (2012) 7323–7329.
[80] C. Qi, T. Bing, H. Mei, X. Yang, X. Liu, D. Shangguan, G-quadruplex DNA aptamers for zeatin recognizing, Biosens. Bioelectron. 41 (2013) 157–162.
[81] F. Gu, L. Zhang, B.A. Teply, N. Mann, A. Wang, A.F. Radovic-Moreno, R. Langer, O.C. Farokhzad, Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 2586–2591.
[82] G. Zhou, O. Latchoumanin, L. Hebbard, W. Duan, C. Liddle, J. George, L. Qiao, Aptamers as targeting ligands and therapeutic molecules for overcoming drug resistance in cancers, Adv. Drug Deliv. Rev. 134 (2018) 107–121.
[83] L.Y. Chou, K. Ming, W.C. Chan, Strategies for the intracellular delivery of nanoparticles, Chem. Soc. Rev. 40 (2011) 233–245.
[84] S. Behzadi, V. Serpooshan, W. Tao, M.A. Hamaly, M.Y. Alkawareek, E.C. Dreaden, D. Brown, A.M. Alkilany, O.C. Farokhzad, M. Mahmoudi, Cellular uptake of nanoparticles: journey inside the cell, Chem. Soc. Rev. 46 (2017) 4218–4244.
[85] S. Laurent, M. Mahmoudi, Superparamagnetic iron oxide nanoparticles: promises for diagnosis and treatment of cancer, Int. J. Molec. Epidemiol. Genet. 2 (2011) 367–390.
[86] V. Mirshafiee, M. Mahmoudi, K. Lou, J. Cheng, M.L. Kraft, Protein corona significantly reduces active targeting yield, Chem. Commun. (Cambridge, England) 49 (2013) 2557–2559.
[87] M. Mahmoudi, N. Bertrand, H. Zope, O.C. Farokhzad, Emerging understanding of the protein corona at the nano-bio interfaces, Nano Today 11 (2016) 817–832.
[88] M. Mahmoudi, I. Lynch, M.R. Ejtehadi, M.P. Monopoli, F.B. Bombelli, S. Laurent, Protein-nanoparticle interactions: opportunities and challenges, Chem. Rev. 111 (2011) 5610–5637.
[89] B.S. Varnamkhasti, H. Hosseinzadeh, M. Azhdarzadeh, S.Y. Vafaei, M. Esfandyari-Manesh, Z.H. Mirzaie, M. Amini, S.N. Ostad, F. Atyabi, R. Dinarvand, Protein corona hampers targeting potential of MUC1 aptamer functionalized SN-38 core–shell nanoparticles, Int. J. Pharm. 494 (2015) 430–444.
[90] D. Ding, Y. Zhang, E.A. Sykes, L. Chen, Z. Chen, W. Tan, The influence of physiological environment on the targeting effect of aptamer-guided gold nanoparticles, Nano Research 12 (2019) 129–135.
[91] A.P. Mann, R.C. Bhavane, A. Somasunderam, B.L. Montalvo-Ortiz, K.B. Ghaghada, D. Volk, R. Nieves-Alicea, K.S. Suh, M. Ferrari, A. Annapragada, D.G. Gorenstein, T. Tanaka, Thioaptamer conjugated liposomes for tumor vasculature targeting, Oncotarget 2 (2011) 298–304.
[92] K. Perschbacher, J.A. Smestad, J.P. Peters, M.M. Standiford, A. Denic, B. Wootla, A.E. Warrington, M. Rodriguez, L.J. Maher 3rd, Quantitative PCR analysis of DNA aptamer pharmacokinetics in mice, Nucleic Acid Therapeut. 25 (2015) 11–19.
[93] C.H. Stuart, R. Singh, T.L. Smith, R. D'Agostino Jr., D. Caudell, K.C. Balaji, W.H. Gmeiner, Prostate-specific membrane antigen-targeted liposomes specifically deliver the Zn(2+) chelator TPEN inducing oxidative stress in prostate cancer cells, Nanomedicine 11 (2016) 1207–1222.
[94] D.B. Kirpotin, D.C. Drummond, Y. Shao, M.R. Shalaby, K. Hong, U.B. Nielsen, J.D. Marks, C.C. Benz, J.W. Park, Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models, Cancer Res. 66 (2006) 6732–6740.
[95] C. Mamot, D.C. Drummond, C.O. Noble, V. Kallab, Z. Guo, K. Hong, D.B. Kirpotin, J.W. Park, Epidermal growth factor receptor–targeted immunoliposomes significantly enhance the efficacy of multiple anticancer drugs < em > In vivo < / em > , Cancer Res. 65 (2005) 11631–11638.
[96] Y. Ma, Y. Jia, L. Chen, L. Ezeogu, B. Yu, N. Xu, D.J. Liao, Weaknesses and pitfalls of using mice and rats in cancer chemoprevention studies, J. Canc. 6 (2015) 1058–1065.
[97] D.M. O'Hanlon, H. Fitzsimons, J. Lynch, S. Tormey, C. Malone, H.F. Given, Soluble adhesion molecules (E-selectin, ICAM-1 and VCAM-1) in breast carcinoma, Eur. J. Cancer 38 (2002) 2252–2257.
[98] N. Ababneh, W. Alshaer, O. Allozi, A. Mahafzah, M. El-Khateeb, H. Hillaireau, M. Noiray, E. Fattal, S. Ismail, In vitro selection of modified RNA aptamers against CD44 cancer stem cell marker, Nucleic Acid Therapeut. 23 (2013) 401–407.
[99] W. Alshaer, H. Hillaireau, J. Vergnaud, S. Ismail, E. Fattal, Functionalizing liposomes with anti-CD44 aptamer for selective targeting of cancer cells, Bioconjug. Chem. 26 (2015) 1307–1313.
[100] S.E. Lupold, B.J. Hicke, Y. Lin, D.S. Coffey, Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen, Cancer Res. 62 (2002) 4029–4033.
[101] A. Bandekar, C. Zhu, R. Jindal, F. Bruchertseifer, A. Morgenstern, S. Sofou, Anti-prostate-specific membrane antigen liposomes loaded with 225Ac for potential targeted antivascular alpha-particle therapy of cancer, J. Nucl. Med. : Off. Publ. Soc. Nucl. Med. 55 (2014) 107–114.
[102] P.J. Bates, E.M. Reyes-Reyes, M.T. Malik, E.M. Murphy, M.G. O'Toole, J.O. Trent, G-quadruplex oligonucleotide AS1411 as a cancer-targeting agent: uses and mechanisms, Biochim. Biophys. Acta Gen. Subj. 1861 (2017) 1414–1428.
[103] P.J. Bates, D.A. Laber, D.M. Miller, S.D. Thomas, J.O. Trent, Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer, Exp. Mol. Pathol. 86 (2009) 151–164.
[104] C.R. Ireson, L.R. Kelland, Discovery and development of anticancer aptamers, Mol. Canc. Therapeut. 5 (2006) 2957–2962.
[105] W.-L. Wan, Z.-X. Liao, E.-Y. Chuang, C.-C. Lin, H.-W. Sung, An aptamer-conjugated liposomal system containing a bubble-generating agent for tumor-specific chemotherapy overcoming multidrug resistance, Nanomed. Nanotechnol. Biol. Med., 12 480.
[106] F. Zhao, J. Zhou, X. Su, Y. Wang, X. Yan, S. Jia, B. Du, A smart responsive dual aptamers-targeted bubble-generating nanosystem for cancer triplex therapy and ultrasound imaging, Small (Weinheim an der Bergstrasse, Germany) 13 (2017).
[107] K. Zhang, M. Liu, X. Tong, N. Sun, L. Zhou, Y. Cao, J. Wang, H. Zhang, R. Pei, Aptamer-modified temperature-sensitive liposomal contrast agent for magnetic resonance imaging, Biomacromolecules 16 (2015) 2618–2623.
[108] T.R. Daniels, E. Bernabeu, J.A. Rodriguez, S. Patel, M. Kozman, D.A. Chiappetta, E. Holler, J.Y. Ljubimova, G. Helguera, M.L. Penichet, The transferrin receptor and the targeted delivery of therapeutic agents against cancer, Biochim. Biophys. Acta 1820 (2012) 291–317.
[109] S.E. Wilner, B. Wengerter, K. Maier, M. de Lourdes Borba Magalhães, D.S. Del Amo, S. Pai, F. Opazo, S.O. Rizzoli, A. Yan, M. Levy, An RNA alternative to human transferrin: a new tool for targeting human cells, molecular therapy, Nucl. Acids 1 (2012) e21.
[110] D.L. Wang, Y.L. Song, Z. Zhu, X.L. Li, Y. Zou, H.T. Yang, J.J. Wang, P.S. Yao, R.J. Pan, C.J. Yang, D.Z. Kang, Selection of DNA aptamers against epidermal growth factor receptor with high affinity and specificity, Biochem. Biophys. Res. Commun. 453 (2014) 681–685.
[111] M.N. Ara, T. Matsuda, M. Hyodo, Y. Sakurai, H. Hatakeyama, N. Ohga, K. Hida, H. Harashima, An aptamer ligand based liposomal nanocarrier system that targets tumor endothelial cells, Biomaterials 35 (2014) 7110–7120.
[112] Y.J. Liu, X.Q. Dou, F. Wang, J. Zhang, X.L. Wang, G.L. Xu, S.S. Xiang, X. Gao, J. Fu, H.F. Song, IL-4Ralpha aptamer-liposome-CpG oligodeoxynucleotides suppress tumour growth by targeting the tumour microenvironment, J. Drug Target. 25 (2017) 275–283.
[113] C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase, Science 249 (1990) 505–510.
[114] E.W. Ng, D.T. Shima, P. Calias, E.T. Cunningham Jr., D.R. Guyer, A.P. Adamis, Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease, Nat. Rev. Drug Discov. 5 (2006) 123–132.
[115] NIH, The Clinical Application of 68Ga Labeled ssDNA Aptamer Sgc8 in Healthy Volunteers and Colorectal Patients, (2018).
[116] NIH, A Study of AS1411 Combined with Cytarabine in the Treatment of Patients with Primary Refractory or Relapsed Acute Myeloid Leukemia, (2018).
[117] G.M. Thurber, M.M. Schmidt, K.D. Wittrup, Antibody tumor penetration: transport opposed by systemic and antigen-mediated clearance, Adv. Drug Deliv. Rev. 60 (2008) 1421–1434.

[118] S.M. Nimjee, C.P. Rusconi, B.A. Sullenger, Aptamers: an emerging class of therapeutics, Annu. Rev. Med. 56 (2005) 555–583.
[119] M. Kim, D.M. Kim, K.S. Kim, W. Jung, D.E. Kim, Applications of cancer cell-specific aptamers in targeted delivery of anticancer therapeutic agents, Molecules 23 (2018).
[120] S.D. Jayasena, Aptamers: an emerging class of molecules that rival antibodies in diagnostics, Clin. Chem. 45 (1999) 1628–1650.
[121] H. Sun, X. Zhu, P.Y. Lu, R.R. Rosato, W. Tan, Y. Zu, Oligonucleotide aptamers: new tools for targeted cancer therapy, Molecular therapy, Nucl. Acids 3 (2014) e182-e182.